JAMES C. NIEDERMAN*
ROBERT B. SCOTT**

Department of Epidemiology and
Public Health, Yale University
School of Medicine.

STUDIES ON INFECTIOUS MONONUCLEOSIS: ATTEMPTS TO TRANSMIT THE DISEASE TO HUMAN VOLUNTEERS†

In spite of the frequency of infectious mononucleosis as a clinical disorder, particularly in the age group of 15 to 25 years, there is a paucity of information regarding the nature of the cause and the routes of transmission of this disease. Many attempts to transmit this disorder in experimental animals inoculated with whole blood, serum, excised lymph nodes, and throat washings from patients with the disease have been conducted without apparent success[1,2] and experimental transmission to man has met only with negative or equivocal results.[3-8] In 1953, Taylor[9] demonstrated heterophile antibody formation in three children having acute leukemia who received pooled sera from patients with infectious mononucleosis. In a subsequent trial, Evans[10] repeated this work by inoculating 2 ml. of pooled sera obtained from patients in the early stages of infectious mononucleosis into a 3½ year old boy with acute leukemia. On the ninth day following inoculation, this patient developed an erythematous rash, fever to 105° F. and leukocytosis with an increase in lymphocytes, but did not have an associated rise in heterophile antibody titer.

Misao and Kobagashi[11] on the other hand reported in 1955 the isolation of *Rickettsia senetsu* from presumed cases of infectious mononucleosis and have described successful transmission of the disease in over 30 human volunteers. Subsequent confirmation of this work has not been reported elsewhere and it has been suggested[12] that the disorder that was transmitted may be an endemic rickettsial disease of Kagami-Fever type.

Recently, electron microscopic studies have revealed that young children with infectious mononucleosis have high concentrations of myxovirus-like particles in their blood similar to those observed in children with acute

leukemia.[18] However, the infectious nature of these particles has not been established at the present time.

Numerous clinical observations, particularly those of Hoagland,[14] suggest that infectious mononucleosis is due to an agent which is presumably present in the throat and blood during early stages of the disease.

The observations to be described in this report have been made during recent attempts to transmit infectious mononucleosis to man. In the present

TABLE 1. RELATION BETWEEN CYTOLOGY OF THROAT SMEARS FROM 568 SUBJECTS AND THEIR SUBSEQUENT DEVELOPMENT OF NINE CASES* OF INFECTIOUS MONONUCLEOSIS WITHIN AN INTERVAL OF 47 DAYS

Group	Characteristics of stained throat smears obtained prior to onset of illness			Total
	Normal lymphocytes present	*Atypical lymphocytes present*	*No white blood cells present*	
Subjects developing infectious mononucleosis	0	1	8*	9
Subjects not developing infectious mononucleosis	15	5	539	559

* Two subjects developed clinical symptoms ten days before throat smears were obtained.

trial, transmission to human volunteers has been attempted by the use of unfiltered material from throat swabs obtained from cases of infectious mononucleosis during the incubation period.

MATERIALS AND METHODS

Two simultaneous pharyngeal swabs were obtained from each of 568 members of an entering freshman class at Smith College, Northampton, Massachusetts. These specimens were collected within 24 hours after the students' arrival at college when they were presumably well; one specimen was immediately placed in a tube containing 2 ml. of Hank's solution and stored at —70° C. in a dry ice chest; a throat smear was made with the other swab which was fixed in methyl alcohol and stained with alkaline methylene blue for microscopic study. Table 1 summarizes the microscopic findings from throat smears of these 568 subjects. Atypical lymphocytes were observed in 6 (1.1 per cent) pharyngeal smears and normal lymphocytes were found in an additional 15 (2.6 per cent). In smears from the remaining 547 persons (96.3 per cent), no white blood cells were present.

Subsequently, nine cases of infectious mononucleosis that fulfilled clinical, hematologic, and serologic criteria were diagnosed in members of this class within six weeks after their entrance into college. As shown in Table 2, 6 of these 9

TABLE 2. CLINICAL AND LABORATORY FINDINGS IN NINE CASES OF INFECTIOUS MONONUCLEOSIS DEVELOPING AT SMITH COLLEGE AMONG 568 WOMEN FROM SEPTEMBER TO NOVEMBER, 1962

| Subject | | No. days between first throat swab and | | Microscopic findings in throat smears | | | | Blood count** | | Heterophile titer after absorption△† | Fever | Remarks |
| No. | Age | Onset of symptoms | Hospital admission | Lymphocytes | | Atypical lymphocytes | | Per cent total lympho-cytes | Per cent atypical lympho-cytes | | | |
				Pre-illness	Post-illness*	Pre-illness	Post-illness*					
1	18	4	5	–	–	–	–	52	8	20	+	Sore throat, macular rash
2	18	5	8	–	–	–	–	87	27	20	+	Sore throat, GGE‡
3	18	9	20	–	+	–	+	81	48	320	+	Headache, cervical adenopathy, enlarged liver and spleen
4	18	16	30	–	–	+	+	50	20	160	+	Sore throat, tonsillar exudate, cervical adenopathy
5	18	24	29	–	+	–	+	80	35	20	+	Sore throat, cervical adenopathy
6	18	26	31	–	+	–	+	66	39	80	+	Headache, sore throat, supra-orbital edema, tonsillar exudate, cervical adenopathy
7	18	47	52	–	–	–	–	70	42	320	+	Headache, cervical adenopathy, enlarged liver and spleen
8	18	10 (after onset of symptoms)	31 (after onset of symptoms)	– (after onset of symptoms)	–	–	–	64	24	80	0	Sore throat, cervical adenopathy
9	18	10 (after onset of symptoms)	27 (after onset of symptoms)	– (after onset of symptoms)	+	–	+	75	20	20	+	Sore throat, tonsillar exudate, cervical adenopathy

* Pre-illness and post-illness = specimens obtained before and during acute illness.
** Maximal changes in white blood cell differential.
† Symbols used follow:

△ = Highest heterophile antibody titer during acute illness. Titer is expressed as reciprocal of serum dilution.
— = Cells absent in stained smear.
+ = Cells present in stained smear.
‡ Generalized glandular enlargement.

subjects developed clinical symptoms of the disease within 4 to 26 days after the initial throat swab had been obtained. In one subject (No. 7) the interval was considerably longer (47 days) and in two other individuals (Nos. 8 and 9), clinical symptoms had apparently begun ten days before collection of the first throat specimen.

In the study of these nine subjects, weekly throat swabs as well as both acute and convalescent serum specimens were obtained during the course of their clinical illness and stored at —70° C. Attention was also directed to the presence of atypical lymphocytes in throat smears obtained after the onset of manifest disease. In 5 of 9 cases, atypical lymphocytes were observed in throat smears obtained after onset of clinical symptoms, and usually were observed during the first two weeks of illness.

Site and subjects

In the transmission trials, eight healthy adult men, ranging in age between 24 and 25 years were studied. These men who had volunteered to be experimental subjects at the Federal Correctional Institution, Danbury, Connecticut, were selected on the basis of age as well as physical condition. Men under the age of 21 years were automatically excluded because they were under the legal age to volunteer for these particular studies.

Initially, a complete medical history was obtained and physical examination was performed on each volunteer in order to exclude those with a history of a past or recent illness characteristic of infectious mononucleosis. Subsequent to the inoculation of presumably infectious material, each subject was examined at least twice weekly and daily temperature readings were obtained for a period of just over six weeks. In order to control the possibility of infection spreading within the institution, volunteers were placed in an isolation unit of the institutional infirmary ten days after inoculation and were hospitalized for a subsequent period of five weeks.

No recognized case of infectious mononucleosis had occurred in this institution for several years prior to these trials and no naturally occurring case was observed there during the course of these studies.

Inoculum

Since it is presumed that the incubation period of infectious mononucleosis may range from approximately 15 to 35 days and that infectivity may be appreciable during the incubation period, unfiltered material was utilized from pooled throat swabs of only those six donors (Nos. 1-6) who developed the disease within 26 days following collection of the "pre-illness" throat swab.

This material, which had been stored at —70° C. for a period of six months prior to inoculation, was thawed just before use. Each of the six specimens was brought to an exact volume of 2.0 ml. using Hank's solution containing penicillin and streptomycin. Each swab was then eluted by forcing media through the cotton with the use of a small syringe. The material from all specimens was then pooled and divided into two equal volumes of 6.0 ml. each. One portion was used for inoculation of four volunteers. The residual volume was stored at —70° C. until used in the second half of the trial.

Approximately 1.0 ml. of inoculum was swabbed directly on the tonsils and posterior pharynx of each volunteer. One half ml. of Hank's solution in which the throat swab had been stored was also introduced directly into the volunteer's nose with a pipette.

Laboratory studies

A total leukocyte count and differential blood count were made on each volunteer before inoculation and twice weekly thereafter for a period of six weeks. Also, a throat swab was obtained and the following laboratory tests were performed prior to inoculation and at weekly intervals thereafter: heterophile antibody test, serum glutamic oxaloacetic transaminase level, direct and total serum bilirubin levels, serum alkaline phosphatase level, cephalin-cholesterol flocculation and thymol turbidity reactions.

RESULTS

Two groups of four adult volunteers were studied sequentially during two separate clinical trials lasting six weeks each. In no instance did any of these eight volunteers develop either sufficient clinical symptoms or objective physical findings that would suggest that transmission of infectious mononucleosis had definitely occurred. As shown in Table 3, slight abnormalities in laboratory tests were demonstrated in 7 of 8 men. However, the development and disappearance of these particular laboratory findings in the volunteers did not seem to follow any regular time pattern following their inoculation.

Three subjects in the first trial developed transient isolated abnormalities in laboratory tests during the 3rd to the 6th week following pharyngeal and intranasal inoculation. *Volunteer 1* developed a serum alkaline phosphatase level of 9.4 units on the 31st day, which declined to 5.8 units during the following week and remained within normal limits thereafter. Although this volunteer's heterophile antibody titer increased from 1:20 to 1:40 before absorption during the 1st and 5th weeks, this increase was not accompanied by any alteration in leukocyte counts, serum transaminase levels, and other liver function tests. On the 26th day following inoculation, *Volunteer 2* had a rise in total lymphocytes to 54 per cent, of which 3 per cent were atypical lymphocytes. The unabsorbed heterophile antibody titer rose from 1:10 to 1:40 during the 5th week, but no associated clinical signs were noted and all laboratory tests were within normal limits one week later. Although *Volunteer 3* developed a leukocytosis of 18,750 during the 3rd week, this white blood count was presumably associated with an infection caused by ingrown nails of both great toes. No other abnormal laboratory abnormalities were observed in this man during the period of observation. *Volunteer 4* was noted to have a slight increase in unabsorbed heterophile titer from negative to 1:20 during the 5th week after inoculation, which then returned to negative during the following and subsequent weeks. In addition, on one occasion early in the 6th week, this volunteer developed 3-plus and 4-plus cephalin-cholesterol

TABLE 3. LABORATORY FINDINGS IN HUMAN VOLUNTEERS DURING ATTEMPTS TO TRANSMIT INFECTIOUS MONONUCLEOSIS

Laboratory finding		First trial Volunteer number				Second trial Volunteer number			
		1 25 yr. Puerto Rican male	2 25 yr. White male	3 25 yr. White male	4 25 yr. White male	5 25 yr. White male	6 24 yr. Negro male	7 25 yr. Puerto Rican male	8 25 yr. White male
White blood count	Pre-inoculation*	7500	8700	11100	7050	7000	8700	9650	8250
	Post-inoculation**	11350	13550	18750	10100	9400	8600	10500	9700
	Day	10	24	17	31	25	—	25	32
Per cent of mononuclears†	Pre-inoculation	36	27	32	32	36	36	44	44
	Post-inoculation	44	54	42	44	56	49	60	49
	Day	26	26	24	45	11	18	18	32
Serum bilirubin (mg./100 ml.)	Pre-inoculation	0.60	0.56	0.60	0.44	0.98	0.74	0.62	0.70
	Post-inoculation	0.60	0.80	0.82	0.68	1.10	0.84	0.70	1.28
	Day	—	45	45	10	25	45	11	18
SGOT‡ (units)	Pre-inoculation	19	23	23	21	24	26	33	28
	Post-inoculation	32	25	28	34	29	44	29	29
	Day	4	26	26	4	25	25	—	39
Alkaline phosphatase (units)	Pre-inoculation	4.9	5.6	6.2	4.4	5.5	7.9	7.6	6.1
	Post-inoculation	9.4	6.4	7.9	8.2	5.6	7.6	8.3	6.6
	Day	31	26	31	17	39	—	39	32
Cephalin flocculation	Pre-inoculation	0	0	0	2+	0	0	0	4+
	Post-inoculation	0	0	0	4+	0	0	1+	4+
	Day	—	—	—	38	—	—	25, 39	—
Thymol turbidity (units)	Pre-inoculation	1.2	3.1	1.8	3.1	2.1	2.2	2.7	3.3
	Post-inoculation	2.3	3.2	2.3	4.9	2.6	3.0	4.1	4.2
	Day	26	4	26	45	32	18	39	39
Heterophile aggluti-nation titer‖	Pre-inoculation	20	10	20	0	20	10	20	40
	Day	4, 38	31	—	31	10	10	10	20
	Post-inoculation	40	40	20	20	—	—	—	—

* Pre-inoculation and Post-inoculation = Highest values before and after inoculation.
** Day = Day on which highest value developed after inoculation.
† Mononuclears = Lymphocytes + Monocytes.
‡ Serum glutamic oxaloacetic transaminase.
‖ Unabsorbed heterophile antibody titer expressed as reciprocal of serum dilution.

flocculation reactions at 24 and 48 hours, respectively. Ten days later, these cephalin-cholesterol flocculation tests were negative, but the thymol turbidity test was elevated to 4.9 units.

All four volunteers in the second trial demonstrated transient abnormal laboratory tests but lacked any associated signs or symptoms of disease. *Volunteer 5* had an increase in lymphocytes from 36 to 56 per cent on the 11th day following inoculation, but no atypical lymphocytes or associated heterophile antibody changes were observed. On the 25th day following inoculation, *Volunteer 6* developed a slightly elevated serum glutamic oxaloacetic transaminase level of 44 units which returned to normal limits one week later and was not associated with other abnormal laboratory tests. During the 2nd, 3rd, and 4th weeks after inoculation, *Volunteer 7* developed an increase in total lymphocytes ranging from 50 to 60 per cent without other hematologic changes. On one occasion during the 6th week after inoculation, this volunteer also developed a slightly elevated thymol turbidity test of 4.1 units, which declined to 2.1 units during the following week. Eighteen days after inoculation, *Volunteer 8* developed a total serum bilirubin of 1.3 mg/100 ml. (0.12 mg/100 ml. direct) which was within normal limits during the 3rd week. This volunteer also demonstrated 3-plus and 4-plus cephalin-cholesterol flocculation reactions repeatedly during this trial, but these abnormalities presumably do not indicate recent hepatic changes since his pre-inoculation tests were 2-plus and 4-plus at 24 and 48 hours, respectively. During the 6th week, this volunteer developed an elevated thymol turbidity of 4.2 units, which decreased to normal limits one week later.

DISCUSSION

In the present trial, use has been made of pooled unfiltered throat swabbings, obtained from six young adult women during various stages of the presumed incubation period of infectious mononucleosis. This material was used to inoculate the throats and intranasal areas of eight adult men in attempts to transmit the disease. Although slight changes, as indicated by transient laboratory abnormalities, did develop in 7 of the 8 volunteers, in no instance was there sufficient supporting evidence to indicate that transmission of the disease had occurred. These findings are similar to the previous attempts of experimental transmission to man by Evans[7,8] and others[4,5,6] who have also described slight equivocal findings such as those reported in this study.

The use of unfiltered throat swabbings as inocula in normal individuals was based on the assumption that early in the incubation period and

perhaps as long as 30-35 days before clinical symptoms develop, an infectious agent is likely to be present in the oropharynx. As shown in Table 1, it was of interest that atypical lymphocytes were observed in 6 of 568 (1.1 per cent) stained throat smears obtained from asymptomatic persons. One of these six women subsequently developed frank clinical signs and symptoms of infectious mononucleosis 16 days later.

Paine[25] has previously described detection of atypical lymphocytes in pharyngeal exudates of patients with full-blown infectious mononucleosis and has commented on their absence in tonsillar and pharyngeal exudates associated with Vincent's angina, diphtheria, and streptococcal infections. In this study, atypical lymphocytes were also detected in throat smears obtained during the first two weeks of clinical symptoms in 4 of 6 patients whose early specimens were used as inocula.

The present work therefore suggests that occasionally atypical lymphocytes may be found in the pharynx early in the incubation period of infectious mononucleosis as well as in later stages of the disease and could be one of the first indications of this disorder.

The lack of successful transmission in these trials may have been due to a variety of circumstances, such as low infectivity of the original inocula or perhaps to destruction of a hypothetical agent by storage and manipulation in the laboratory. Since only adult male volunteers, aged 24 to 25 years, were utilized in this study, another pertinent factor is the relative degree of susceptibility of men of this age to the disease. The age distribution of many previously reported cases suggests that this is primarily an infectious disease of children and young adults.[10,16] If the natural history of infectious mononucleosis is similar to that of certain viral infections, the ratio of inapparent to apparent infections may be quite high and the former may enormously outnumber the latter. It is even conceivable that in children and young adults as in poliomyelitis, perhaps only as few as 1 or 2 out of 100 infections exhibit frank clinical, hematologic, and serologic changes, whereas, 1 in 10 or 20 infections may represent a subclinical infection characterized by a febrile disorder associated with an abnormal blood picture, but without any serologic changes. The infrequency of multiple cases in roommates has often been cited as a reason for supporting such a view.

According to this schema, the majority of clinical infections would be either inapparent or subclinical, *i.e.,* associated with only slight fever and such few clinical symptoms that the disorder would not be recognized as infectious mononucleosis. In the present study then, it may be that either all of these particular eight adult volunteers had previously experienced natural inapparent infections and were resistant to reinfection, or that

all or some of them were actually susceptibles, but had sustained inapparent infections. According to the theory of Evans,[10,16] which maintains that a positive heterophile antibody test only occurs in the severe clinical cases of infectious mononucleosis, some of these volunteers may have suffered from such equivocal illness, although it is evident that this is only speculation. One thing is clear in the present study, however, namely, that no recognized case of infectious mononucleosis occurred in the course of these particular attempts to transmit the disease.

SUMMARY

Experimental transmission of infectious mononucleosis has been attempted in eight adult male volunteers, ranging in age between 24 and 25 years. The inocula consisted of pooled throat swabbings obtained from six young adult women between 4 and 26 days prior to the onset of infectious mononucleosis. In addition to clinical, hematologic, and serologic evidence of infectious mononucleosis in these donors, atypical lymphocytes were demonstrated in stained throat smears obtained from one subject during the incubation period and in 4 of 6 women during the course of their illnesses.

Although transient abnormalities in laboratory findings were observed in 7 of 8 volunteers, none of these adult volunteers developed convincing evidence of successful transmission of this disorder.

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